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Prostaglandins, lysosomes, and radiation injury

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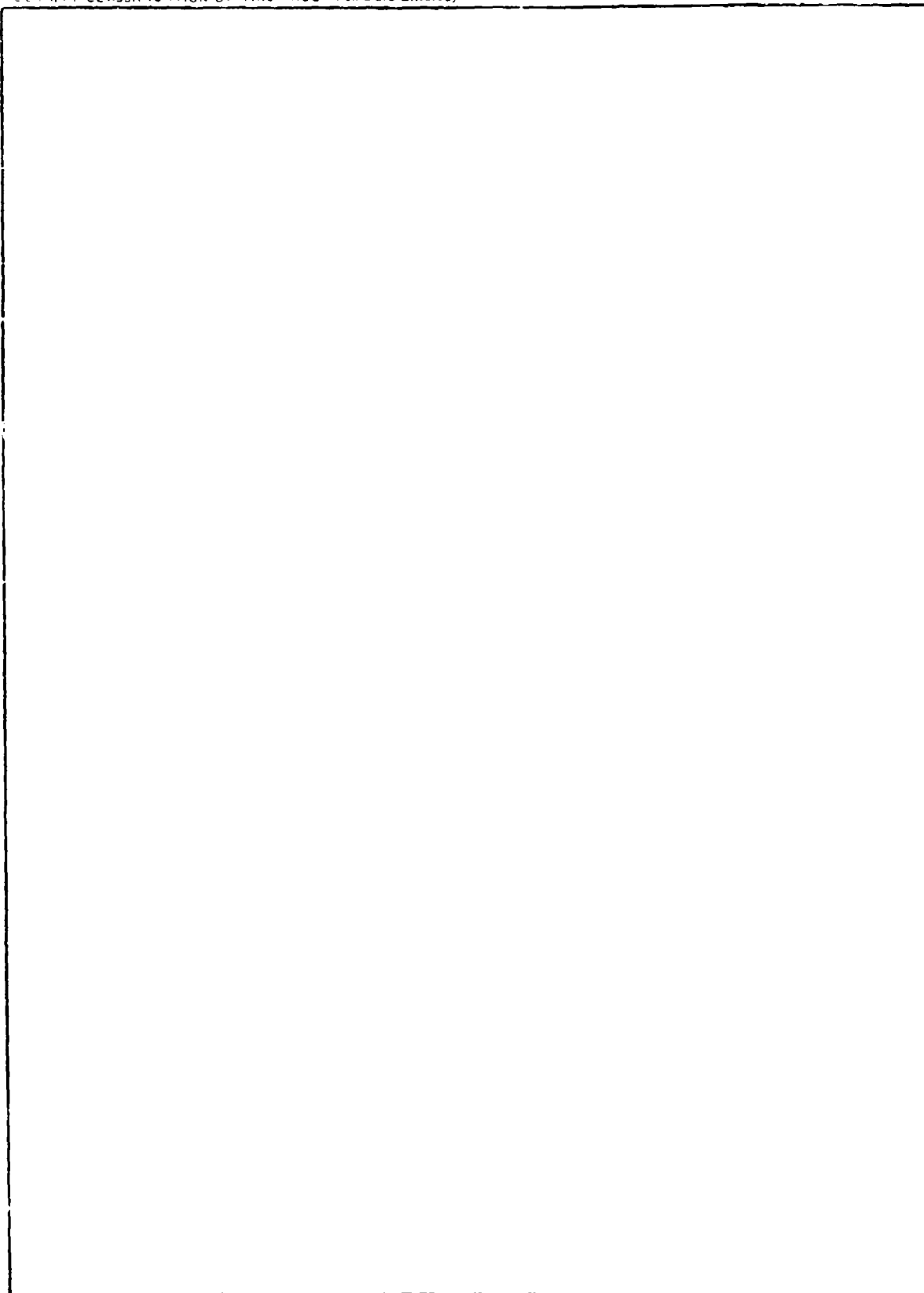
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Prostaglandins, Lysosomes, and Radiation Injury

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Prostaglandins (PGs) and lysosomal enzymes have been shown to be altered with tissue injury (3). Yet no studies have been performed to monitor lysosomal enzyme activities and PG levels simultaneously in animals exposed to ionizing radiation. Therefore, the present investigation was designed to determine the relationship between lysosomal enzymes and PG levels in irradiated tissues.

MATERIALS AND METHODS

Sprague-Dawley rats (75–125 g) were exposed bilaterally to 1,000 rad of gamma radiation at a dose of 500 rad/min. At designated time intervals after irradiation, the rats were anesthetized with ether and exsanguinated through the auxillary artery. Spleen and liver tissues were rapidly excised and frozen with liquid nitrogen except for 0.05- to 0.1-g portions of each fresh tissue sample. The fresh spleen and liver aliquots were homogenized in 0.2 M KCl with a Dounce homogenizer. A portion of each homogenate was centrifuged at $12,000 \times g$ in order to obtain a supernatant without of lysosomes. The remaining portions of the tissue homogenates were frozen and thawed twice. The uncentrifuged tissue homogenates and the $12,000 \times g$ supernatants were then assayed for β -glucuronidase activity (1).

Isolation of PGs from the frozen tissues were performed by the extraction and purification techniques described in Fig. 1. Analysis of PGE_1 and $PGF_{2\alpha}$ content was performed by immunoassay (4).

RESULTS

β -Glucuronidase Levels

Within 6 hr after irradiation, the levels of rat spleen β -glucuronidase activity began to increase, reaching a maximal activity in 4 days that was 3 times greater than control levels (Fig. 2). However, the spleen percent supernatant activity of β -glucuronidase displayed a different pattern than the total β -glucuronidase activity. Within 5 to 12 hr after irradiation, the percentage of β -glucuronidase activity in the $12,000 \times g$ spleen supernatant increased from an average value

TISSUE (0.5 - 4.0 gm) FROZEN IN LIQUID NITROGEN HOMOGENIZED IN 30 - 40 ml EtOH (CONTAINING ^3H PG) AND CENTRIFUGED

→ PRECIPITATE DISCARDED

EtOH EVAPORATED UNDER VACUUM AT 45°C. RESIDUE DISSOLVED IN 0.5 M TRIS, pH 7.5 (1.5 ml) AND PARTITIONED WITH EtAc (2.0 ml) TWICE.

→ EtAc DISCARDED

AQUEOUS PHASE MADE pH 4.5 WITH 0.5 M CITRATE, pH 4.0 (0.5 ml) AND PARTITIONED WITH EtAc (3.0 ml) TWICE.

→ AQUEOUS DISCARDED

POOLED EtAc WASHED WITH H_2O (0.2 ml) AND EVAPORATED UNDER VACUUM AT 37°C. RESIDUE DISSOLVED IN 2.5 % MeOH IN CHCl_3 (0.2 ml) AND FRACTIONATED ON 0.6 x 9 cm SILICIC ACID COLUMN:

FRACTION 1 (DISCARDED) 2 ml OF CHCl_3 + 2 ml OF 2.5 % MeOH IN CHCl_3

FRACTION 2 (PG A) 8 ml OF 2.5 % MeOH IN CHCl_3

FRACTION 3 (PG E) 10 ml OF 5.0 % MeOH IN CHCl_3

FRACTION 4 (PG F) 6 ml OF 20 % MeOH IN CHCl_3

FIG. 1. Scheme for isolating PGs from tissue.

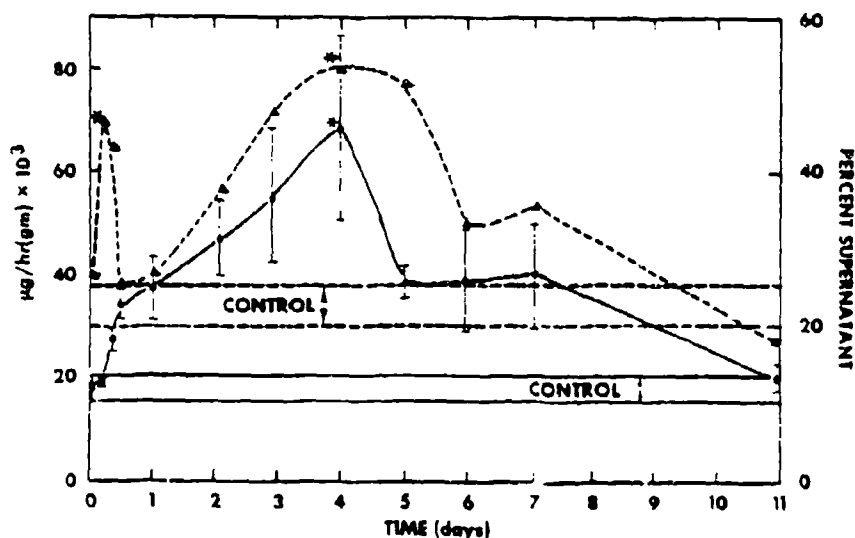


FIG. 2. Effect of ^{60}Co gamma radiation on the concentration of spleen β -glucuronidase in rats for unfractionated spleen homogenate activity (—) and of percent supernatant activity ($12,000 \times g$ β -glucuronidase activity/unfractionated β -glucuronidase homogenate activity $\times 100$) (---). Means \pm SD are shown, where SD is indicated by vertical lines. Means significantly different (*) from control range (control \pm SD); $p < 0.01$.

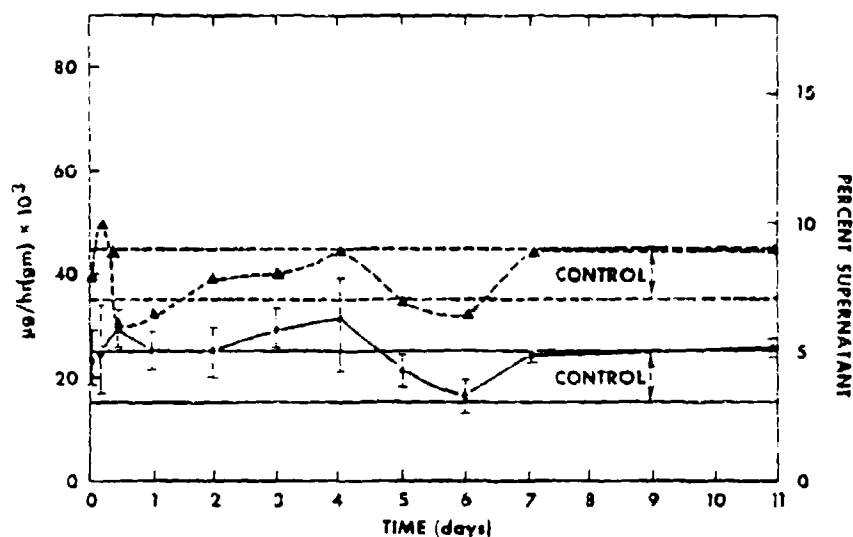


FIG. 3. Effect of ^{60}Co gamma radiation on the concentration of liver β -glucuronidase in rats for unfractionated liver homogenate activity (—) and of percent supernatant activity (---). For explanation of means and SD, see legend to Fig. 2.

of $22 \pm 2\%$ to a significant $47 \pm 6\%$ ($p < 0.001$). Again, after 3 to 5 days, the soluble β -glucuronidase activity in the supernatant increased to over 50% of the total activity before subsiding to a normal value at 11 days.

As compared with spleen β -glucuronidase levels, only slight variations in liver β -glucuronidase activity were observed in rats irradiated with ionizing radiation (Fig. 3). No significant increases in liver β -glucuronidase levels were observed in rat liver tissue after irradiation. Also, there were no significant increases in the percentage of liver β -glucuronidase activities found in the 12,000 \times g supernatant as compared with controls.

PG Levels

Gamma ray exposure of liver and spleen tissues caused a significant increase in $\text{PGF}_{2\alpha}$ levels (Figs. 4 and 5). Within 5 to 12 hr postirradiation, $\text{PGF}_{2\alpha}$ levels displayed transient increases in both spleen and liver tissues. Later, at 4 and 7 days, spleen and liver $\text{PGF}_{2\alpha}$ levels rose markedly.

Unlike the $\text{PGF}_{2\alpha}$ value in spleen tissue (Fig. 4), there was no transient increase in the spleen PGE_1 value several hours after exposure of rats to ionizing radiation (Fig. 6). There were, however, large increases in PGE_1 levels in spleen tissue on 4 and 7 days postirradiation.

DISCUSSION

The large increases in spleen β -glucuronidase activities at 5 to 12 hr, 3 to 4 days, and 6 to 7 days after irradiation of rats by gamma rays have been observed

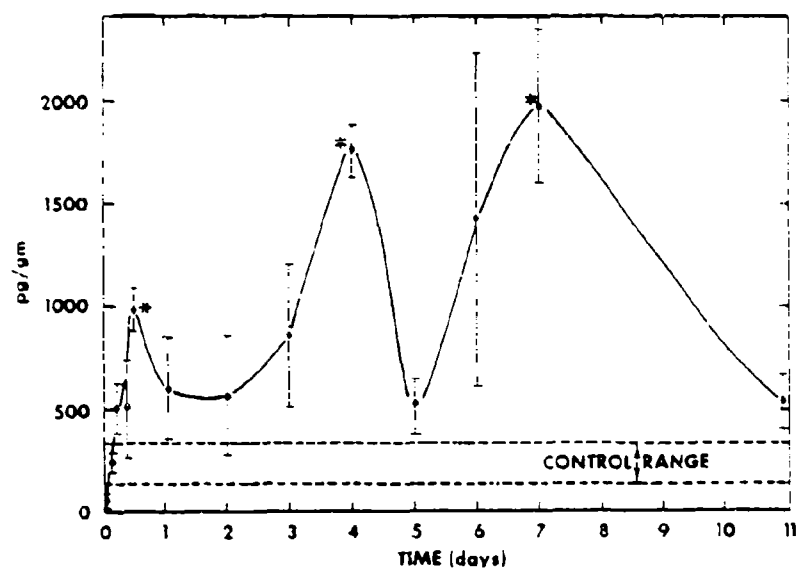


FIG. 4. Effect of ^{60}Co gamma radiation on the concentration of spleen $\text{PGF}_{2\alpha}$ in rats. See legend to Fig. 2 for explanation of means, SD, and p values.

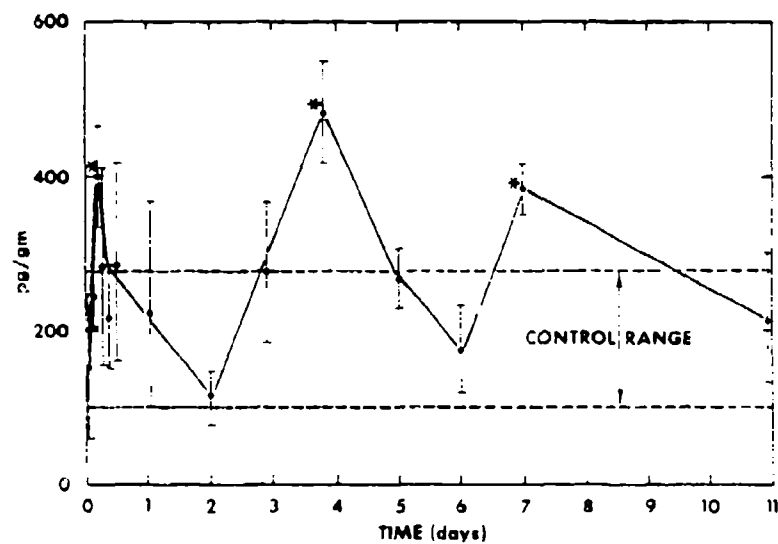


FIG. 5. Effect of ^{60}Co gamma radiation of the concentration of liver $\text{PGF}_{2\alpha}$ in rats. See legend to Fig. 2 for explanation of means, SD, and p values.

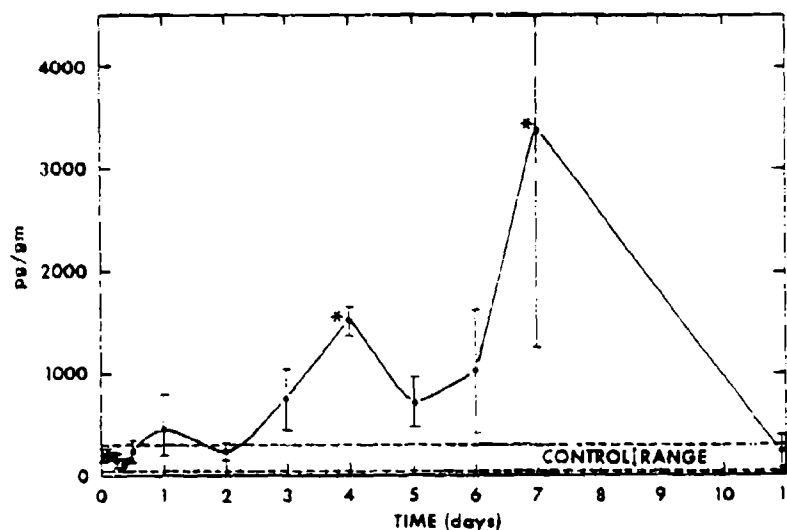


FIG. 8. Effect of ^{60}Co gamma radiation of the concentration of spleen PGE_1 in rats. See legend to Fig. 2 for explanation of means, SD, and p values.

by other investigators (6,7). As indicated in Fig. 3 and shown by Snyder (6) and Watkins (7), slight increases in liver β -glucuronidase activity also occurred at 5 to 12 hr, 3 to 4 days, and possibly 6 to 8 days, following exposure of animals to ionizing radiation. The leakage of β -glucuronidase from both liver and spleen lysosomes occurred at nearly the same time as the total β -glucuronidase activity increased in spleen and liver tissues.

Like the lysosomal enzyme β -glucuronidase, the PGs reached their maximal levels at 5 to 12 hr for $\text{PGF}_{2\alpha}$, 3 to 4 days for PGE_1 and $\text{PGF}_{2\alpha}$, and 6 to 7 days for PGE_1 and $\text{PGF}_{2\alpha}$ after rats had been irradiated. Eisen (2) and Păulescu et al. (5) have also reported increases in PGE and PGF levels in spleen and liver tissues at 4 or 7 days postirradiation, but their PG concentrations were 30 to 200 times larger than the physiological values reported in here. However, due to the isolation methods used by Eisen (2) and Păulescu et al. (5), nonspecific synthesis of PGs would have occurred, resulting in an elevation of their PGE_1 and $\text{F}_{2\alpha}$ levels.

Changes in PG levels and β -glucuronidase activities occurred at nearly the same times and with similar magnitudes. These changes indicate that either the increase in lysosomal enzyme activities and leakage of lysosomes, cause PG levels to rise, or an elevation in PG concentration may result in the activation and release of enzymes from the lysosomes.

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